Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

methyl *N*-(isopropoxycarbonyl)-L-valyl-(3*RS*)-3-(4-chlorophenyl)-β-alaninate; valifenalate

EC Number: -
CAS Number: 283159-90-0

CLH-O-0000006928-58-01/F

Adopted

10 December 2020

10 December 2020

CLH-O-0000006928-58-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **Valifenalate**

EC Number: **-**

CAS Number: **283159-90-0**

The proposal was submitted by **Hungary** and received by RAC on **14 November 2019.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Hungary has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **3 February 2020**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **3 April 2020**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Miguel A. Sogorb**

Co-Rapporteur, appointed by RAC: **Anja Menard Srpčič**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **10 December 2020** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	methyl *N*-(isopropoxycarbonyl)-L-valyl-(3*RS*)-3-(4-chlorophenyl)-β-alaninate; valifenalate		283159-90-0	Aquatic Chronic 2	H411	GHS09	H411			
RAC opinion	TBD	methyl *N*-(isopropoxycarbonyl)-L-valyl-(3*RS*)-3-(4-chlorophenyl)-β-alaninate; valifenalate		283159-90-0	Carc. 2 Aquatic Chronic 2	H351 H411	GHS08 GHS09 Wng	H351 H411			
Resulting Annex VI entry if agreed by COM	TBD	methyl *N*-(isopropoxycarbonyl)-L-valyl-(3*RS*)-3-(4-chlorophenyl)-β-alaninate; valifenalate		283159-90-0	Carc. 2 Aquatic Chronic 2	H351 H411	GHS08 GHS09 Wng	H351 H411			

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Valifenalate (methyl *N*-(isopropoxycarbonyl)-L-valyl-(3*RS*)-3-(4-chlorophenyl)-β-alaninate) is a new active substance in the meaning of Regulation (EU) No 1107/2009 developed as fungicide. It has no previous entry in Annex VI of Regulation EC 1272/2008.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The Dossier Submitter (DS) proposed no classification of valifenalate for physical hazards based on the following facts:

- Negative results with an EEC A.14 assay for testing explosive properties;
- Negative results with an EEC A.10 assay for testing flammability;
- Negative results with an EEC A.16 assay for testing self-heating; and,
- Negative results with an EEC A.17 assay for testing oxidising properties.

No data for the following hazards were provided by the DS:

- self-reactivity,
- pyrophoricity,
- capability to emit flammable gases and
- corrosivity to metals.

Comments received during consultation

No comments were received during consultation.

Assessment and comparison with the classification criteria

RAC notes that no test for explosivity was found in the CLH-report since Annex I shows that the A.14 test report was limited to a prediction based on structure. Nevertheless, the molecule of valifenalate does not contain groups associated with explosive properties and therefore no test is needed. Thus, **RAC supports no classification for explosivity.**

With regard to flammability, RAC notes that a preliminary test according to A.10 (equivalent to a preliminary test according to UN N.1) was negative. Thus, RAC supports **no classification for flammability.**

The result of the A.16 test was negative. However, RAC notes that the A.16 test is not the same as that required under CLP criteria (UN N.4) for testing self-heating. Therefore, RAC supports **no classification for self-heating but in this case, due to inconclusive data.**

No test was available for assessing the oxidising capability of valifenalate. However, RAC notes that the molecule contains oxygen and chlorine, but these are bonded only to carbon and therefore no test is need. Thus, **RAC supports no classification for oxidising properties.**

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification of valifenalate based on OECD-guideline and GLP compliant tests showing an LD_{50} higher than 5000 mg/kg bw for the oral route and higher than 2000 mg/kg bw for the dermal route, and an LC_{50} higher than 3.1 mg/l for the inhalation route.

Comments received during consultation

One manufacturer/company agreed with the DS's proposal for no classification.

Assessment and comparison with the classification criteria

Table 1 summarised all the available studies for assessment of acute toxicity of valifenalate.

***Table 1**: Summary of animal studies on acute toxicity with valifenalate.*

Study	Dose level	Results	Reference
Acute oral toxicity OECD TG 401 GLP Sprague Dawley rats (Crl: CD (SD) BR) 5/sex/group	Valifenalate (IR5885) Purity: 98.9% 5000 mg/kg bw Single dose followed by 14 days observation	No mortalities Transient piloerection in all animals the day after treatment No appreciable macroscopic changes in necropsies of treated animals LD_{50} > 5000 mg/kg bw	Confidential study number 62
Acute dermal toxicity OECD TG 402 GLP Sprague Dawley rats (Crl: CD (SD) BR) 5/sex/group	Valifenalate (IR5885) Purity: 98.6% 2000 mg/kg bw 24 h dermal exposure followed by 14 days observation	No mortalities No clinical effects No local irritation No appreciable macroscopic changes in necropsies of treated animals LD_{50} > 2000 mg/kg bw	Confidential study number 63
Acute inhalation toxicity OECD TG 403 GLP Wistar Han-Ibm rats 5/sex/group	Valifenalate (IR5885) Purity: 98.6% MMAD: 2.42, 2.45 µm GSD: 2.95, 2.89 Gravimetric concentration: 3.118 mg/l 4 hour nose-only exposure of an aerosol followed by 14 days observation	No mortalities No significant signs of toxicity Slight reduction in body weight between days 1 and 4 No macroscopic changes at termination LC_{50} > 3.118 mg/L air (gravimetric mean aerosol concentration) (highest technically achievable concentration)	Confidential study number 11

Comparison with the criteria

The cut-off point for triggering classification for both acute oral and acute dermal toxicity is 2000 mg/kg bw. Table 1 shows as two reliable OECD-guideline studies conducted observing GLP procedures yielded LD_{50} values higher than 5000 and 2000 mg/kg bw for oral and dermal toxicity; respectively. Thus, RAC supports the DS's proposal for **no classification of valifenalate for acute oral and dermal toxicity.**

The cut-off point for triggering classification for acute inhalation toxicity of dusts and aerosols is 5 mg/l. Table 1 shows as one reliable OECD-guideline study conducted observing GLP procedures yielded an LC_{50} higher than the maximum achievable concentration (3.1 mg/L). Thus, RAC supports the DS's proposal for **no classification of valifenalate for acute inhalation toxicity.**

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

The DS proposed no classification of valifenalate based on the absence of specific effects reported in the acute toxicity tests (see Table 1) and the absence of neurotoxicity in one acute neurotoxicity study using doses up to 2000 mg/kg bw.

Comments received during consultation

One manufacturer/company agreed with the DS's proposal for no classification.

Assessment and comparison with the classification criteria

Comparison with the criteria

RAC notes the absence of organ specific effects in the acute studies via oral, dermal and inhalation routes (Table 1). The CLH-report presents also an acute neurotoxicity study performed in rats conducted following OECD TG 424 and observing GLP. On this study, 10 rats/sex/group were treated with single doses of 500, 1000 and 2000 mg/kg bw of valifenalate (purity 98.9%) in 0.5% w/v methylcellulose in water. Animals were further observed for 14 days. Doses lower than 2000 mg/kg bw caused no effects on rats. The top dose (2000 mg/kg bw) caused a slight incidence of axonal degeneration in multiple nerves but without observing a clear dose-response.

Overall, none of the single-dose animal studies contained in the CLH-report provided evidence of organ-specific toxicity; which prevents for classification as STOT SE Cat 1 or 2. Moreover, no narcotic effects or respiratory tract irritation were found in such studies; which prevents for classification as STOT SE Cat 3. Therefore, RAC supports the DS's proposal for **no classification of valifenalate as STOT SE.**

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS proposed no classification for skin irritation based on a dermal irritation study showing no signs of irritation in 3/3 New Zealand rabbits.

Comments received during consultation

One manufacturer/company agreed with the DS's proposal for no classification.

Assessment and comparison with the classification criteria

Table 2 summarises the findings in the skin corrosion/irritation study available in the CLH-report.

Table 2: *Summary of the animal study on skin corrosion/irritation with valifenalate.*

Study	Dose level	Results	Reference
Acute dermal irritation OECD TG 404 GLP New Zealand White rabbits 3 males	Valifenalate (IR5885) Purity: 98.6% 0.5 g/animal Single 4 hour application Application sites scored at: 1, 24, 48 and 72 hours after patch removal (Draize scheme)	No signs of irritation <u>Mean scores / animal (24, 48 & 72 hours):</u> Erythema: 0, 0, 0 Oedema: 0, 0, 0	Confidential study number 33

Comparison with the criteria

RAC notes that the skin irritation study performed according to OECD TG 404 and GLP showed that valifenalate was not able to irritate skin of rabbits since no erythema and no oedema was found in any of the three treated New Zealand White rabbits (Table 2). Thus, RAC supports the DS proposal for **no classification of valifenalate for skin irritation/corrosion.**

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS proposed no classification for eye damage/irritation based on an eye damage study showing light conjunctival redness 1 hour after instillation (fully reversible by 24 hours) but no signs of corneal or iris damage and no signs of conjunctival redness or chemosis by 24 hours and thereafter.

Comments received during consultation

One manufacturer/company agreed with the DS's proposal for no classification.

Assessment and comparison with the classification criteria

Table 3 summarises the findings in the acute eye irritation/corrosion study available in the CLH-report.

Table 3: *Summary of the animal study on eye irritation/corrosion with valifenalate**.***

Study	Dose level	Results	Reference
Acute Eye Irritation/Corrosion OECD TG 405 GLP New Zealand White rabbits	Valifenalate (IR5885) Purity: 98.6% 0.1 g/animal Single instillation Eyes scored at: 1, 24, 48 and 72 hours	Slight (grade 1), conjunctival redness was seen at the 1 hour examination in 3/3 rabbits (fully reversed by 24 hours)	Confidential study number 34

3 males	after instillation	Mean Scores / animal (24, 48 & 72 hours): Cornea: 0, 0, 0, Iris: 0, 0, 0, Conjunctiva redness: 0, 0, 0. Conjunctiva chemosis: 0, 0, 0.

Comparison with the criteria

RAC notes that only grade 1 conjunctival redness was seen 1 hour after instillation while no signs of eye damage was seen by 24 hours and thereafter in an OECD TG 405 study conducted observing GLP (Table 3). Thus, RAC supports the DS proposal for **no classification of valifenalate for eye damage/irritation.**

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

The DS proposed no classification of valifenalate for respiratory sensitisation based on lack of data.

Comments received during consultation

One company manufacturer commented that the conclusion of lack of data is not correct since test for respiratory sensitisation cannot be provided because no formally recognised and validated animal test currently exists. The DS thanked the comment and replied that this hazard was not in the scope of the public consultation, although the provided comments will be brought to consistency with the conclusion.

Assessment and comparison with the classification criteria

Comparison with the criteria

RAC notes that: i) there are no data indicating evidence of respiratory tract irritation with valifenalate; ii) the acute inhalation study showed no evidence of respiratory system impairment; and iii) rabbit dermal and eye irritation studies indicated lack of irritant potential on skin and mucosal membranes. Overall, RAC supports the DS's proposal for **no classification of valifenalate for respiratory sensitisation.**

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS proposed no classification of valifenalate for skin sensitisation based on the negative result of a guinea pig maximisation test conducted following OECD TG 406 and observing GLP.

Comments received during consultation

One manufacturer/company agreed with the DS's proposal for no classification.

Assessment and comparison with the classification criteria

Table 4 summarises the findings in the skin sensitisation study available in the CLH-report.

***Table 4**: Summary of the animal study on skin sensitisation with pyridalyl*.

Study	Dose level	Results	Reference
Maximisation test OECD TG 406 GLP Dunkin Hartley guinea pigs 17 males (10 test, 5 controls, 2 preliminary test)	Valifenalate (IR5885) Purity: 98.6% Vehicle: corn seed oil **Induction:** Intradermal: 1% in corn seed oil, 1% in Freund's Complete Adjuvant (FCA) and FCA emulsion (1:1 v/v FCA/water)-day 0. Topical: Pre-treatment with 0.5 ml 10% sodium lauryl sulfate in Vaseline oil-day 5. Test article (10%) or vehicle applied under an occlusive dressing for 48 hours. **Challenge** Test article (10%) and vehicle applied to the flanks of all animals under an occlusive dressing for 24 hours.	**Induction** Slight, swollen reddish seen 24 hours after the intradermal injections with FCA and /or test material. There were no signs of irritation observed following the topical induction. **Challenge:** Challenge sites assessed at 24 and 48 hours. No dermal reaction following challenge in test or control animals. **No positive reactions at 24 and 48 hours. Sensitisation rate: 0%** **Positive control (2-mercaptobenzothiazole):** Sensitisation rate 40%.	Confidential study number 47

Comparison with the criteria

The guinea pig maximisation test conducted according to OECD TG 406 Guideline and observing GLP showed no evidence that valifenalate is a dermal sensitiser. RAC notes that the question whether higher concentrations could have been tested using other vehicles remains unresolved and gives uncertainties for the assessment. Overall, RAC supports the DS's proposal for **no classification of valifenalate for skin sensitisation.**

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The repeated dose toxicity studies with animals showed that valifenalate is able to cause small changes in blood and clinical chemistry parameters as well as hepatocyte hypertrophy in rats, increased relative liver weight and centrilobular hepatocyte hypertrophy in mice, and an increase in alkaline phosphatase (ALP) and hepatocyte hypertrophy in dogs. The DS noted that these effects were generally seen at doses above the guidance cut-off values and were of low severity (i.e. the alterations in blood and clinical chemistry). Other changes (centrilobular hypertrophy and associated increases in liver weight and in the activity of ALP) were considered adaptive in response to administration of valifenalate. The DS proposed no classification of the substance for STOT RE.

Comments received during consultation

One manufacturer/company agreed with the DS's proposal for no classification.

Assessment and comparison with the classification criteria

Tables 5, 6 and 7 summarise the results of the repeated dose toxicity studies in rats, mice and dogs; respectively.

Table 5: *Summary of repeated dose toxicity studies in rats with valifenalate*. In all cases the effects were statistically different from controls for at least $p<0.05$. ND = No statistical differences with control.

Method	Results	Reference
28-day oral toxicity study Based on OECD TG 407 (1995) but no GLP compliance claimed Preliminary study for a 90 day Non GLP Oral (continuous in diet) Rat Han Wistar 5/sex/group Valifenalate (IR5885) Purity: 98.9% 0, 120, 600, 3000 and 15000 ppm Vehicle: laboratory animal diet	No treatment-related deaths in any dose group <u>15000 ppm (1518/1537 mg/kg bw/day males/females)</u> **males / females** ↓ Body weight gain weeks 0-4: 25% / ND- ↓ Food consumption weeks 0-4: 12% / 10% ↓ Haematocrit: 5% / 4% ↓ Haemoglobin: 5% / 4% ↓ Total lymphocyte count: 22% / 34% ↑ Activated partial thromboplastin time: 23% / ND ↑ Aspartate aminotransferase activity: ND / 24% ↓ Calcium: 3% / 5% ↓ Phosphorous: - / 21% ↓ Total protein: 3% / 7% ↑ A/G ratio: - / 7% ↓ Absolute thymus weight: 32% / 14% Thymic lymphocytosis (always slight grade): 2/5 vs 0/5 controls / 4/5 vs 2/5 controls <u>3000 ppm (311/314 mg/kg bw/day males/females)</u> **males / females** ↓ Haematocrit: 10% / ND ↓ Total lymphocyte count: 11% / 33% ↓ Calcium: 4% / 5% ↓ Phosphorous: - / 19% ↓ Total protein: 3% / 9% ↑ A/G ratio: ND / 13% ↓ Absolute thymus weight: ND / 14% Thymic lymphocytosis (always slight grade): 4/5 vs 0/5 controls / ND <u>600 ppm (63/64 mg/kg bw/day males/females)</u> **males / females** ↓ Haematocrit: ND / 5% ↓ Haemoglobin: 4% / ND ↓ Calcium: 4% / 5% ↓ Phosphorous: ND / 15% ↓ Total protein: 3% / 6% ↑ A/G ratio: ND / 9% Thymic lymphocytosis (always slight grade): 3/5 vs 0/5 controls / ND <u>120 ppm (13 mg/kg bw/day males & females)</u>	Confidential study number 48

No adverse effects.

Conclusion:
NOAEL: 311 mg/kg bw/day
LOAEL: 1518 mg/kg bw/day

90-day oral toxicity study

4 week recovery period

OECD GT 408 (1998)

GLP

Oral (continuous in diet)

Rat

Han Wistar

10/sex/group

5/sex/control & high dose groups for recovery phase

Valifenalate (IR5885)

Purity: 98.9%

0, 7, 150, 1000 mg/kg bw/day

Vehicle: laboratory animal diet

There were no deaths or overt signs of toxicity in any dose group.

Confidential study number 49

<u>1000 mg/kg bw/day</u>

	males	females
↓ Haematocrit	5%	ND
↓ Haemoglobin	4%	ND
↓ Red blood cell	2%	ND
↓ White blood cell	13%	ND
↓ Monocyte count	28%	ND
↑ Platelet count	7%	ND
↓ Prothrombin time	10%	ND
↓ Neutrophil count	ND	31%
↓ Triglycerides	36%	ND
↑ Chloride	2%	ND
↑ Calcium	ND	3%
↑ Urine volume	60%	68%
↓ Specific gravity	ND	1039 g/l vs 1050 g/l control
↑ pH	7.3 vs 6.9 controls	6.4 vs 5.9 controls
↑ Relative liver weight	15%	13%
Distended caecum	7/10 vs 0/10 controls	1/10 vs 0/10 controls

<u>150 mg/kg bw/day</u>

	males	females
↓ Haematocrit	2%	ND
↓ Haemoglobin	3%	ND
↓ White blood cell	24%	ND
↓ Monocyte count	24%	ND
↑ Platelet count	11%	ND
↓ Prothrombin time	8%	ND
↓ Triglycerides	34%	ND
↑ Chloride	1%	ND
↑ pH	7.3 vs 6.9 controls	6.4 vs 5.9 controls

<u>7 mg/kg bw/day</u>

	males	females
↑ pH	7.3 vs 6.9 controls	ND

Recovery from all treatment-related effects occurred in the 4 weeks recovery period.

Conclusion:
NOAEL: 150 mg/kg bw/day
LOAEL: 1000 mg/kg bw/day

52-week chronic toxicity (from 2 year study)

OECD TG 453 (1981)

GLP

Oral (continuous in diet)

Rat

Han Wistar

20/sex/group

Valifenalate (IR5885)

Purity: 99.56%

0, 15, 150, 1000 mg/kg bw/day

Vehicle: laboratory animal diet

<u>1000 mg/kg bw/day</u>

	males	females
↓ Body weight	9%	ND
↓ Haemoglobin	2.5-3.8%	ND
↓ Red cell count and mean cell haemoglobin concentration	1.4-3.5%	ND
↑ Platelet count	9-16%	10%
↑ APTT time	19-28%	ND
↑ Urine volume	ND	75-210%
↓ Specific gravity	1035-1041 g/l vs 1047-1066 g/l controls	ND
↑ Relative liver weights	19%	12%
↑ Relative kidney weights	8%	ND
Thyroid follicular cell hypertrophy	10 slight + 1 moderate vs 3 slight controls	ND

<u>150 mg/kg bw/day</u>

	males	females
↓ Mean cell haemoglobin concentration	1.7%	ND
Thyroid follicular cell hypertrophy	5 slight vs 3 slight controls	ND

<u>15 mg/kg bw/day</u>

	males	females
Thyroid follicular cell hypertrophy	2 slight vs 3 slight controls	ND

Conclusion:
NOAEL: 150 mg/kg/day
LOAEL: 1000 mg/kg/day

Confidential study number 51

28-day dermal toxicity study

OECD TG 410 (1981)

GLP

Dermal (6 hours/day)

Rat

Han Wistar

10/sex/group

Valifenalate (IR5885)

Purity: 99.6%

0, 1000 mg/kg bw/day

Vehicle: sterile water

No treatment-related effects

Conclusion:
NOEL: 1000 mg/kg bw/day

Confidential study number 23

Two generation reproduction (one litter)

OECD TG 416 (2001)

GLP

Oral (continuous in diet)

Rat

HanBrl:WIST

Valifenalate (IR5885)

Purity: 99.56%

0, 1250, 4300 or 15000 ppm (reduced to 0, 850, 2900 or 10000 ppm during lactation)

Vehicle: laboratory animal diet

Parental toxicity

Confidential study number 27

15000 ppm (10000 ppm) – 986/1150 mg/kg bw/day, males/ females (P generation - pre-pairing)

	P		F1	
	male	female	male	female
↑ Absolute liver weight	16%	15%	12%	8%
↑ Relative liver weight	20%	11%	14%	10%
Hepatocellular hypertrophy	15/24 (severity 2.4) vs 4/24 (severity 1.3) controls	3/24 (severity 2.0) vs 0/24 controls	21/24 (severity 2.2) vs 2/24 (severity 2.0) controls	21/24 (severity 1.9) vs 0/24 controls
Glycogen deposition liver	17/24 (severity 1.3) vs 21/24 (severity 1.6) controls	15/24 (severity 1.3) vs 15/24 (severity 2.3) controls	19/24 (severity 1.5) vs 23/24 (severity 2.7) controls	2/24 (severity 1.0) vs 13/24 (severity 1.8) controls
Ruffled fur early lactation	ND	ND	ND	4/24
↓ Absolute kidney weight	ND	ND	ND	7%
↓ Relative kidney weight	ND	ND	ND	6%
Thyroid follicular hypertrophy	ND	ND	22/24 (severity 2.1) vs 17/24 (severity 1.4) controls	19/24 (severity 1.6) vs 10/24 (severity 1.1) controls

4300 ppm (2900 ppm) – 277/318 mg/kg bw/day, males/ females (P generation - pre-pairing)

	P		F1	
	male	female	male	female
↑ Absolute liver weight	6%	ND	6%	ND
↑ Relative liver weight	9%	ND	8%	ND
Hepatocellular hypertrophy	7/24 (severity 1.3) vs 4/24 (severity 1.3) controls	ND	17/24 (severity 2.3) vs 2/24 (severity 2.0) controls	ND
Glycogen deposition liver	17/24 (severity 1.3) vs 21/24 (severity 1.6) controls	17/24 (severity 1.8) vs 15/24 (severity 2.3) controls	23/24 (severity 1.9) vs 23/24 (severity 2.7) controls	7/24 (severity 1.4) vs 13/24 (severity 1.8) controls
Ruffled fur early lactation	ND	ND	ND	4/24

Thyroid follicular hypertrophy	ND	ND	16/24 (severity 1.8) vs 4/24 (severity 1.3) controls	16/24 (severity 1.8) vs 17/24 (severity 1.7) controls

1250 ppm (850 ppm) – 80/92 mg/kg bw/day, males/ females (P generation - pre-pairing)

No treatment related effects in both P and F1 generations

Conclusion:
NOAEL parental toxicity: 80 mg/kg bw/day
LOAEL parental toxicity: 318 mg/kg bw/day

The 28-days, 90-days and 53-weeks repeated dose toxicity studies in rats showed that valifenalate was able to induce minor changes in blood and clinical chemistry (Table 5). Although these changes were consistent among different studies, the severity is relatively low. The CLH-report provides historical control data (HCD) showing that the minor differences between treated and control animals were of no toxicological relevance because the records of the altered parameters were within the HCD. Therefore, RAC notes that the changes in blood and clinical chemistry found in the repeated dose toxicity studies in rat do not support a classification as STOT RE.

The repeated dose toxicity studies in rat suggest that thymus is a potential target organ of valifenalate. Indeed, decreases in absolute thymus weight and increases in thymic lymphocytosis were used for setting the LOAEL of the 28-days repeated dose toxicity study (Table 5).

Thyroid follicular cell hypertrophy was reported in the 52-weeks repeated toxicity study and in the F1 generation of the 2-generation reproduction toxicity study (Table 5), although in the latter the meaning of this effect is unclear because no clear dose response was found and high background incidence was noted. Overall, RAC notes that these thyroid effects could support a potential classification as STOT RE.

The incidence of distended caecum was also clearly increased in males versus controls in the 90-days repeated dose toxicity study (Table 5). The toxicological significance of this effect is still unclear but RAC notes that it could support a potential classification as STOT RE.

The repeated dose toxicity studies in rat suggest that also liver is a target organ of valifenalate. Increases in liver weight were reported in the 90-days, 52-weeks and 2-generation oral toxicity studies (Table 5). RAC notes that these increases in liver weight were moderate and can be an adaptive response to valifenalate administration and therefore cannot be considered for setting classification as STOT RE. A dose-dependent hepatocellular hypertrophy was reported in both P and F1 generations in the 2-generation study (Table 5). However, liver hypertrophy is cited in the Guidance on the Application of the CLP Criteria as an adaptive (compensatory) response that is generally reversible with no adverse consequences on cessation of exposure. Thus, the observed liver hypertrophy does not warrant classification as STOT RE.

Glycogen deposition in liver was reported in the 2-generation toxicity study (Table 5). However, RAC notes that no clear dose-response was observed and there was also a high incidence in control groups. Thus, the observed glycogen deposition in liver does not warrant a potential classification as STOT RE.

Table 6**:** *Summary of repeated dose toxicity studies in mice with valifenalate***.** In all cases the effects were statistically different from controls for at least $p<0.05$. ND = No statistical differences with control.,

Method	Results	Reference
28-day oral toxicity study Based on OECD TG 407 (1995) but no compliance claimed Preliminary study for a 90 day Valifenalate (IR5885, batch no. FCF/T/180-00 (ex ZI068) Purity: 98.9% 0, 110, 440, 1750 and 7000 ppm Vehicle: laboratory animal	7000 ppm (1105/1536 mg/kg bw/day males/females) ↓ Haematocrit: males 10%, females ND ↓ Haemoglobin: males 11%, females ND ↓ Red blood cell: males 10%, females ND ↑ Glucose: males 39%, females 31% ↓ Triglycerides: males ND, females 71% ↑ Cholesterol: males 31%, females ND ↑ Potassium: males 15%, females 19% ↓ Sodium: males ND, females 2% ↓ Chloride: males ND, females 3% ↓ Total protein: males ND, females 10% ↓ Albumin: males ND, females 7% ↑ A/G ratio: males ND, females 4% ↑ Relative liver weight: males 52%, females 41% ↑ Relative adrenal weights: males 45%, females ND Centrilobular hepatocytic hypertrophy: males 4 slight + 2 moderate vs 0/6 controls, females 5 (slight) vs 1 slight control 1750 ppm (266/402 mg/kg bw/day males/females) ↓ Haematocrit: males 4%, females ND ↓ Haemoglobin: males 6%, females ND ↓ Red blood cell: males 5%, females ND ↑ Glucose: males 38%, females 32% ↓ Triglycerides: males ND, females 44% ↑ Potassium: males ND, females 2% ↓ Chloride: males ND, females 3.5% ↓ Total protein: males ND, females 4% ↓ Albumin: males ND, females 3% ↑ A/G ratio: males ND, females 2% ↑ Relative liver weight: males 31%, females 14% Centrilobular hepatocytic hypertrophy: males 6 slight vs 0/6 controls, females 2 moderate vs 1 slight control 440 ppm (68/96 mg/kg bw/day males/females) ↑ Relative liver weight: males ND, females 10% Centrilobular hepatocytic hypertrophy: males 6 slight vs 0/6 controls, females ND 110 ppm (18/27 mg/kg bw/day males/females) No treatment-related effects **Conclusion:** **NOAEL: 68 mg/kg bw/day** **LOAEL: 266 mg/kg bw/day**	Confidential study number 48
90-day oral toxicity study Based on OECD TG 408 (1998) but no	7000 ppm (995/1144 mg/kg bw/day males/females) ↓ Body weight gain weeks 0-13: males 26%, females ND ↓ Haematocrit: males 4%, females 5% ↓ Haemoglobin: males 4%, females 3%	Confidential study number 50

compliance claimed	↓ Mean cell haemoglobin	5%	3%
	↓ Mean cell volume	5%	4%
Prelim carcinogenicity study	↑ Relative liver weight	51%	35%
	Centrilobular hepatocellular vacuolation	4 slight + 4 moderate vs 1 minimal + 1 slight controls	ND
GLP	Periportal hepatocellular vacuolation	1 minimal + 1 slight + 1 moderate vs 0/10	ND

Oral (continuous in diet)

Mouse

CD-1

10/sex/group

Valifenalate (IR5885, batch no. FCF/T/180-00 (ex ZI068)

Purity: 98.9%

0, 110, 900 and 7000 ppm

Vehicle: laboratory animal diet

<u>900 ppm (133/147 mg/kg bw/day males/females)</u>

	males	females
Relative liver weight	12%	ND

<u>110 ppm (15/16 mg/kg bw/day males/females)</u>

No treatment-related effects

Conclusion:
NOAEL: 133 mg/kg bw/day
LOAEL: 995 mg/kg bw/day

Carcinogenicity (1.5-year) study

OECD TG 451

Mouse

Crl: CD-1™ (ICR) BR

50/sex/group

Valifenalate (IR5885)

Purity: 99.56%

0, 150, 850, 5000 ppm

Continuous dietary administration for 78 weeks

Achieved doses 16.8, 97.2 and 657 mg/kg/day for males and 21.6, 124 and

Non-neoplastic findings

<u>5000 ppm (657/756 mg/kg bw/day)</u>

	males	females
↓ Body weight	22%	ND
↑ Relative liver weight	97%	23%
↑ Relative kidney weight	ND	12%
↑ Centrilobular hepatocyte hypertrophy	ND	22 slight + 3 moderate vs 5 slight + 2 moderate + 1 marked controls
Generalised hepatocyte hypertrophy	18 slight + 11 moderate vs 3 slight controls	ND
Centrilobular hepatocyte vacuolation	11 slight + 20 moderate + 1 marked vs 3 minimal + 7 slight + 1 moderate controls	ND
Cytoplasmic eosinophilia in hepatocytes	29/50 vs 0/50 controls	ND
Pigment in hepatocytes	18/50 vs 0/50 controls	13/50 vs 0/50 controls

Confidential study number 52

756 mg/kg/day for females	Pigment in hepatocyte macrophages	12/50 vs 1/50 controls	31/50 vs 12/50 controls
	Gall bladder choleliths	ND	8/45 vs 1/47

850 ppm (97.2/124 mg/kg bw/day)

	males	females
↑ Relative liver weight	29%	ND
Centrilobular hepatocyte vacuolation	2 minimal + 11 slight + 22 moderate vs 3 minimal + 7 slight + 1 moderate controls	ND

150 ppm (17/22 mg/kg bw/day)

	males	females
Centrilobular hepatocyte vacuolation	21 slight + 13 moderate vs 3 minimal + 7 slight + 1 moderate controls	ND

Conclusion:
NOAEL: 17 mg/kg bw/day
LOAEL: 97 mg/kg bw/day

The effects reported in mice (Table 6) were consistent with the effects reported in rats (Table 5). Moderate alterations of blood and clinical values were reported in the 28-days and 90-days repeated toxicity studies (Table 6). The incidence of these alterations were relatively moderate and, in concordance with changes reported in rats, RAC does not consider these effects enough robust for supporting a STOT RE classification.

The studies in mice also highlight liver as target organ of valifenalate. Moderate increases in relative liver weight (up to 50%) were noted in the 28-days and 90-days repeated dose toxicity studies (Table 6). This increase was more notable (around 100%) in the carcinogenicity study (Table 6). Histopathological alterations in liver were noted in several studies in mice. These alterations include mainly hepatocyte hypertrophy and vacuolation, cytoplasmic eosinophilia and hepatocyte and macrophage pigmentation (Table 6). RAC notes that all these changes in liver are indeed adaptive responses by the same reason outlined in the case of rat studies and therefore should be considered for setting classification as STOT RE.

Other effects were also described in these repeated dose toxicity studies in mice as 45% increase in adrenal weight, 12% increase in relative kidney weight and increases in incidences of gall bladder choleliths (Table 6). However, RAC notes that these effects were not consistently reported among different studies in mice and were not noted in rat and dog studies and therefore RAC does not consider these effects for classification as STOT RE.

***Table 7**: Summary of repeated dose toxicity studies in dogs with valifenalate*. In all cases the effects were statistically different from controls for at least p<0.05. ND = No statistical differences with control.,

Method	Results	Reference
28-day oral toxicity study OECD TG 409 (1998) GLP Oral (capsule) Dog Beagle 3/sex/group Valifenalate (IR5885) Purity: 98.9 0, 250, 500 and 1000 mg/kg bw/day Vehicle: gelatine capsule	<u>1000 mg/kg bw/day</u> See table below <u>500 mg/kg bw/day</u> See table below	Confidential study number 7

	males	females
↑ Pale faeces	3/3	2/3
↓ Cholesterol	60%	67%
↓ Phospholipid	53%	61%
↑ Alkaline phosphatase	203%	ND
↑ Gamma glutamyl-transferase	80%	ND
↑ Total protein	13%	18%
↓ Albumin	20%	23%
↓ Calcium	8%	11%
↓ Magnesium	10%	ND
↑ Phosphorous	18%	ND
↑ Absolute liver weight	66%	33%
Hepatocellular glycogen content	0/3 vs 2/5 (severity 2.5) controls	1/3 (severity 1.0) vs 3/3 (severity 3.0) controls
Hepatocellular hypertrophy	3/3 (severity 4.0) vs 1/3 (severity 1.0) controls	3/3 (severity 3.3) vs 0/3 controls
Liver eosinophilic cytoplasmic inclusions	3/3 (severity 2.3) vs 0/3 controls	2/3 (severity 3.0) vs 0/3 controls
Liver single cell necrosis	3/3 (severity 1.0) vs 0/3 controls	1/3 (severity 0.33) vs 0/3 controls
Liver apoptosis	1/3 (severity 0.6) vs 0/3 controls	ND

	males	females
↑ Pale faeces	3/3	ND
↓ Cholesterol	41%	52%
↓ Phospholipid	38%	44%
↑ Total protein	9%	14%
↓ Albumin	18%	21%
↓ Calcium	ND	10%
↑ Absolute liver weight	49%	42%
Hepatocellular glycogen content	3/3 (severity 2.0) vs 2/3 (severity 2.5) controls	3/3 (severity 2.0) vs 3/3 (severity 3.0) controls

Hepatocellular hypertrophy	3/3 (severity 3.0) vs 1/3 (severity 1.0) controls	3/3 (severity 2.7) vs 0/3 controls
Liver eosinophilic cytoplasmic inclusions	3/3 (severity 2.0) vs 0/3 controls	2/3 (severity 1.5) vs 0/3 controls

250 mg/kg bw/day

	males	females
↓ Cholesterol	42%	19%
↓ Phospholipid	40%	ND
↑ Total protein	8%	ND
↓ Albumin	23%	ND
Hepatocellular glycogen content	3/3 (severity 2.7) vs 2/3 (severity 2.5) controls	3/3 (severity 1.3) vs 3/3 (severity 2.0) controls
Liver eosinophilic cytoplasmic inclusions	2/3 (severity 1.5) vs 0/3 controls	1/3 (severity 1.0) vs 0/3 controls

Conclusion:
NOAEL: 500 mg/kg bw/day
LOAEL: 1000 mg/kg bw/day

90-day oral toxicity study

OECD TG 409 (1998)

GLP

Oral (capsule)

Dog

Beagle

4/sex/group

Valifenalate (IR5885)

Purity: 98.56%

0, 50, 250 and 750 mg/kg bw/day

Vehicle: gelatine capsule

750 mg/kg bw/day

1 female taken off-dose after 7 weeks due to weight loss adverse laboratory results and retained until the end of the study

White discoloured faeces or white/yellow powder in faeces from day 3, 7/8 dogs

	males	females
↓ Body weight gain	48%	33%
↓ Food consumption	12%	12%
↑ Platelets	Up to 33%	Up to 74%
↓ RBC	8%	9%
↑ MCH	9%	10%
↑ MCV	7%	ND
↓ Reticulocytes	50%	Up to 60%
↑ ALP	Up to 517%	Up to 446%
↑ ALT	Up to 109%	Up to 303%
↑ GGT	Up to 133%	Up to 133%
↓ Cholesterol	Up to 60%	Up to 69%
↓ Total protein	Up to 18%	Up to 17%
↓ Albumin	Up to 23%	Up to 28%
↑ AST	28%	24%
↑ Glucose	12%	22%
↑ Relative liver weight	60%	70%
↑ Relative thyroid/parathyroid weights	64%	ND

Confidential study number 12

↓ Prostate weight	64%	ND
↓ Testis weight	28%	ND
↑ Epididymis weight	14%	ND
Hepatocyte hypertrophy	4 moderate vs 0/4 controls	3 moderate vs 0/4 controls
Hepatocytes pale cytoplasm, peripheral clumping	2 slight + 2 moderate vs 0/4 controls	3 moderate vs 0/4 controls
Eosinophilic intracytoplasmic inclusions in hepatocytes	2 slight + 2 moderate vs 0/4 controls	1 slight + 2 moderate vs 0/4 controls
Thyroid follicular hypertrophy	1 minimal + 1 slight vs 0/4 controls	2 minimal vs 0/4 controls

250 mg/kg bw/day

↑ white discoloured faeces or white/yellow powder in faeces from day 10, 5/8 dogs

	males	females
↓ Body weight gain	21%	ND
↑ Platelets	Up to 42%	ND
↓ Reticulocytes	31%	39%
↑ ALP	Up to 430%	Up to 194%
↑ ALT	ND	42%
↑ GGT	33%	33%
↓ Cholesterol	Up to 47%	Up to 36%
↓ Total protein	Up to 13%	Up to 11%
↓ Albumin	Up to 20%	Up to 13%
↑ AST	ND	29%
↑ Relative liver weight	44%	34%
↑ Relative thyroid/parathyroid weights	61%	ND
Hepatocyte hypertrophy	2 slight + 2 moderate vs 0/4 controls	1 minimal + 1 slight + 2 moderate vs 0/4 controls
Hepatocytes pale cytoplasm, peripheral clumping	2 slight + 2 moderate vs 0/4 controls	1 minimal + 1 slight + 2 moderate vs 0/4 controls
Eosinophilic intracytoplasmic inclusions in hepatocytes	2 slight + 2 moderate vs 0/4 controls	3 minimal + 1 slight vs 0/4 controls
Thyroid follicular hypertrophy	1 minimal vs 0/4 controls	2 slight vs 0/4 controls

50 mg/kg bw/day

	males	females
↑ ALP	Up to 142%	Up to 134%
↑ Relative liver weight	-	33%
Hepatocyte hypertrophy	3 minimal + 1 slight vs 0/4 controls	2 minimal + 2 slight vs 0/4 controls
Thyroid follicular hypertrophy	ND	1 slight vs 0/4 controls

Conclusion:
NOAEL: 250 mg/kg bw/day
LOAEL: 750 mg/kg bw/day

52-week chronic toxicity

Additionally 13 weeks sub-chronic toxicity with 8 week recovery

OECD TG 452 (1981)

GLP

Oral (capsule)

Dog

Beagle

4/sex/group

Valifenalate (IR5885)

Purity: 99.56%

0, 1, 7, 50 and 250 mg/kg bw/day

Vehicle: gelatine capsule

250 mg/kg bw/day

	males	females
↑ Platelets	Up to 74%	ND
↑ ALP	Up to 1360%	Up to 746%
↓ Cholesterol	28%	25%
↓ Total protein	Up to 13%	Up to 10%
↓ Albumin	Up to 19%	Up to 16%
↑ Triglycerides	91%	ND
↓ Calcium ions	Up to 8%	ND
↑ Relative liver weight	61%	36%
↑ Relative thyroid/parathyroid	31%	ND
↓ Relative prostate weight	29%	ND
↓ Relative ovary weights	ND	57%
Hepatocyte hypertrophy	3 slight + 1 moderate vs 0/4 controls	3 slight + 1 moderate vs 0/4 controls
Hepatocytes with pale cytoplasm and peripheral clumping hypertrophy	4 minimal vs 0/4 controls	3 minimal vs 0/4 controls

50 mg/kg bw/day

	males	females
↑ ALP	Up to 217%	Up to 398%
↓ Relative ovary weights	-	48%
Hepatocyte hypertrophy	2 minimal + 2 slight vs 0/4 controls	3 minimal + 1 slight vs 0/4 controls
Hepatocytes with pale cytoplasm and peripheral clumping hypertrophy	ND	1 minimal vs 0/4 controls

7 mg/kg bw/day

	males	females
↑ ALP	165%	150%
Hepatocyte hypertrophy	1 minimal vs 0/4 controls	1 minimal + 1 slight vs 0/4 controls

Confidential study number 65

1 mg/kg bw/day

	males	females
↑ ALP	ND	55%

Conclusion:
NOAEL: 50 mg/kg bw/day
LOAEL: 250 mg/kg bw/day

The database with dogs shows a scenario consistent with information obtained with rats and mice. Alterations in clinical and blood chemistry were noted in the three available studies. However, most of these changes were of low magnitude; the largest changes reported were the high increase of transaminase activities (ALP and ALT) (Table 7). RAC notes that the changes in transaminases are secondary to liver response and therefore should not be considered as supporting for classification as STOT RE.

The assessment of the dog studies shows again the liver as target organ of valifenalate. Indeed, increases in relative liver weight, hepatocellular hypertrophy and liver eosinophilic cytoplasmic inclusions were consistently reported through the whole database. Again, as in the case of rats and mice, RAC noted that at exposure levels below the guidance values, these changes are adaptive responses rather than adverse effects and therefore cannot be used as basis for supporting a classification. However, RAC notes certain incidences of liver single cell necrosis in the 28-day study. On the opposite to hypertrophy, necrosis is a non-reversible event that might notably alter the performance of liver and therefore should be taken into consideration for classification as STOT RE.

Some changes were noted in reproductive organs (reductions in prostate, testis and ovary weight and increases in epididymis weight) (Table 7). However, these alterations will be assessed within the reproductive toxicity hazard class and not for STOT RE. The thyroid, in the mice studies, exhibited certain alterations after valifenalate exposure. These changes were mainly reduction in relative thyroid/parathyroid and thyroid follicular hypertrophy (Table 7). However, RAC noted that these effects were not reported in all studies and no dose-response was observed in the case of thyroid follicular hypertrophy (Table 7). Overall, RAC does not consider the effects in thyroid robust enough for supporting a potential classification as STOT RE.

Comparison with the criteria

Table 8 summarises all findings of Tables 5, 6 and 7 on adverse effects relevant for STOT-RE classification that were consistently observed in available repeated toxicity studies.

Table 8: *Adverse effects of valifenalate relevant for STOT-RE classification.* **Bolded text** refers to those effects that appear at doses relevant for classification as STOT RE.

Effect	Study	Lowest reported dose (mg/kg bw/day)	Guidance value for STOT-RE classification Cat 1/Cat 2 (mg/kg bw/day)
↓ Absolute thymus weight, thymic lymphocytosis, distended caecum	28-day study (rats)	1518	30/300
Thyroid follicular cell hypertrophy	52-week (rats)	1000	2.5/25
Thyroid follicular cell hypertrophy	2-generation reproduction (rats)	277	8.9/89 (assuming 112 days of exposure)
Liver single cell necrosis	28-days study (dogs)	1000	30/300

Table 8 shows as none of the effects considered for supporting a classification as STOT RE appear at concentrations within the corresponding guidance values. Therefore, RAC supports **no classification of valifenalate for STOT RE** based on the observed effects.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification of valifenalate for germ cell mutagenicity based on three *in vitro* and one *in vivo* negative studies.

Comments received during consultation

One company-manufacturer agreed with the DS's proposal for no classification.

Assessment and comparison with the classification criteria

Tables 9 and 10 summarise the results of the mutagenicity/genotoxicity assays contained in the CLH-report.

Table 9: *Summary of mutagenicity/genotoxicity in vitro studies with valifenalate***.**

Method	Tested concentrations	Results	Reference
In vitro bacterial gene mutation Ames test OECD TG 471 GLP Strains: TA98, TA100, TA102, TA1535, TA1537 of Salmonella typhimurium	Valifenalate (IR5885) Purity: 98.9% Positive controls: sodium azide; 4-nitro-o-phenylene-diamine; methyl methane sulfonate and 2-aminoanthracene Solvent: Dimethyl sulfoxide (DMSO) Concentrations : 33, 100, 333, 1000, 2500 and 5000 valifenalate µg/plate	**+S9: Negative** **-S9: Negative**	Confidential study number 53
In vitro clastogenicity in mammalian cells Chromosome aberration test OECD TG 473 GLP Chinese Hamster Ovary (CHO/D1) cells	Valifenalate (IR5885) Purity: 98.9% Positive controls: ethylmethane sulfonate and cyclophosphamide Solvent: Dimethylsulfoxide (DMSO) Concentrations : Experiment 1: Concentrations of up to 1600 µg /mL (with and without S9 mix) Experiment 2: Concentrations of up to 200 µg /mL (without S9 mix) and up to 1600 µg /mL (with S9 mix)	**+S9: Negative** **-S9: Negative**	Confidential study number 41
In vitro mammalian gene mutation OECD TG 476 GLP L5178Y mouse lymphoma cells	Valifenalate (IR5885) Purity: 98.9% Positive controls: 3-methyl chloranthracene and methyl methane sulfonate Solvent: Dimethyl sulfoxide (DMSO) Concentrations :	**+S9: Negative** **-S9: Negative**	Confidential study number 54

	Experiment 1: 12.5, 25, 50, 100, 200 and 400 µg/mL (with and without S9 mix) Experiment 2: 25, 50, 100, 200, 400 & 800 µg/mL (without S9 mix)		

Table 10: *Summary of the mutagenicity/genotoxicity in vivo study with valifenalate.*

Method	Tested concentrations	Results	Reference
In vivo mouse micronucleus OECD TG 474 GLP NMRI mouse 6/sex/group	Valifenalate (IR5885) Purity: 99.56% Positive control: cyclophosphamide Vehicle: corn oil 24 hours preparation interval groups dosed at: 0, 500, 1000 or 2000 mg/kg bw valifenalate plus positive control group 48 hours preparation interval: an additional group dosed at 2000 mg/kg bw	**Negative**	Confidential study number 20

Comparison with the criteria

The genotoxicity of valifenalate was tested in three *in vitro* and one *in vivo* tests. The results of all studies were negative with positive and negative controls demonstrating the validity of the tests. Thus, RAC supports the DS's proposal for **no classification of valifenalate for germ cell mutagenicity.**

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The CLH-report contains two carcinogenicity studies. That in rats showed no neoplastic findings, while the study in mice showed increased incidence over control and historical control data (HCD) of hepatocellular adenomas in both sexes at 850 and 5000 ppm and increases of hepatocellular carcinoma in males at 5000 ppm. The CLH-report also provides some mechanistic studies for demonstrating that the carcinogenicity in mouse liver is triggered by a mechanism based on a key event consisting in activation of multiple nuclear receptors, followed by a key event consisting in an increase in the DNA replicative synthesis which, in turn, is followed by the last key event, consisting in the formation of the hepatocellular injury. The DS proposed no classification of valifenalate for carcinogenicity based on the lack of relevance for humans of the proposed mechanism of action.

Comments received during consultation

One MSCA questioned the results and conclusions derived from the confidential study number 69 on the basis of: i) inappropriate comparison between strains; ii) a weak induction of peroxisome proliferator-activated receptor (PPAR-α) in the knock-out model; and iii) lack of positive control in this experiment. This same MSCA also questioned the lack of experiments with constitutive androstane receptor (CAR)/pregnane X receptor (PXR) knockout mice in the database in order to clarify the role of these receptors in the hepatocarcinogenesis. Finally, the MSCA also questioned why valifenalate was not able to activate nuclear receptors while positive controls did. Overall, this MSCA considered the receptor activation by valifenalate to be demonstrated but not the lack of relevance for humans because alternative mechanisms of action were not addressed

and they therefore supported classification as Carc. 2 H351. The DS replied to these comments as follows:

- Providing an additional historical control data (HCD) from Charles River Laboratories showing that hepatocellular adenoma incidences in males were almost covered and the incidence in females were covered by these new HCD records.
- Highlighting the arguments presented in Annex 2 of the CLH report (and summarised below; see "Supplemental Information") and considering that: i) the "Bradford Hill Considerations" of the WHO International Programme on Chemical Safety support the proposed mechanism of action based on nuclear receptor activation; ii) the lack of relevance for humans, since neither CAR/PXR nor the PPAR-α are regarded as relevant to humans; and iii) evidences that carcinogenicity in liver in this case is not based on alternative mechanisms of action such as genotoxicity, cytotoxicity, aryl hydrocarbon receptor (AhR)- or oestrogen receptor (ER)-mediated mechanism.

One company-manufacturer supported the DS's proposal for no classification.

Assessment and comparison with the classification criteria

A summary of the information contained in the Annex 2 of the CLH-report entitled "*Valifenalate: Mode of Action Analysis using the WHO/IPCS Mode of Action Framework*" is presented in the Background Document.

Table 11 summarises the results of the two carcinogenicity studies found in the CLH-report.

Table 11: *Summary of carcinogenicity studies with valifenalate*.

Method	Results	Reference
2-year combined toxicity and carcinogenicity study OECD TG 453 GLP Rat HsdBrl Han Wistar 50/sex/group: 104 weeks 20/sex/group: 52 weeks Valifenalate (IR5885) Purity: 99.56% 0,15,150, 1000 mg/kg bw/day Continuous dietary administration	**Non-neoplastic findings** See Table 5 for effects at 52 weeks <u>Effects at week 104: 1000 mg/kg bw/day</u> No effects on body weight, haematology and urine analysis Increases of relative liver weight of 9.9% ($p<0.01$) (males) and 7.6% ($p<0.01$) (females) <u>Effects at week 104: 150 mg/kg bw/day</u> Reduction of 8% in male body weight <u>Effects at week 104: 15 mg/kg bw/day</u> No toxicologically significant treatment-related effects **Neoplastic findings** **No treatment-related changes in neoplastic findings at any dose level**	Confidential study number 51
Carcinogenicity study	**Non-neoplastic findings** See Table 6	Confidential study number 52

OECD TG 451

Mouse

Crl: CD-1™ (ICR) BR

50/sex/group

Valifenalate (IR5885)

Purity: 99.56%

0, 150, 850, 5000 ppm mg/kg bw/day

Continuous dietary administration for 78 weeks

Achieved doses 16.8, 97.2 and 657 mg/kg/day for males and 21.6, 124 and 756 mg/kg/day for females

Neoplastic findings

Males

	Dietary concentration (ppm)				
	0	150	850	5000	HCD (%)[$]
No. Examined	50	50	50	50	-
Hepatocellular Adenoma (%)	7 (14)	2 (4)	14 (28)	16* (32)	7.8-21.2
Hepatocellular carcinoma (%)	2 (4)	4 (8)	4 (8)	10* (20)	1.9-8.0
Combined adenoma + carcinoma** (%)	9 (18)	6 (12)	18 (36)	26 (52)	-
*p ≤ 0.05 compared with control group **Estimated by RAC, not provided by the DS, no available statistical analysis [$] No contextual information about this HCD was provided					

Females

	Dietary concentration (ppm)				
	0	150	850	5000	HCD (%)[$]
No. Examined	50	50	50	50	
Hepatocellular adenoma	0 (0)	0 (0)	2 (4)	5* (10)	0.0-1.9
Hepatocellular carcinoma	0 (0)	1 (2)	0 (0)	0 (0)	0.0-0.0
Combined adenoma + carcinoma**	0 (0)	1 (2)	2 (4)	5 (10)	
*p ≤ 0.05 compared with control group **Estimated by RAC, not provided by the DS, no available statistical analysis [$] No contextual information about this HCD was provided					

In Han Wistar rats there was no evidence of valifenalate-related carcinogenicity up to and including the limit dose level for carcinogenicity studies of 1000 mg/kg/day (Table 11). In CD-1 mice valifenalate induced hepatocellular adenomas and carcinomas in males. The incidence of these tumours in males and females given 850 or 5000 ppm exceeded the background range in studies performed at this facility (Table 11). For males, at 850 ppm the incidence of adenoma and carcinoma was 28 and 8% respectively, and at 5000 ppm the incidences were 32 and 20%, respectively. The incidences of adenomas exceeded the historical control range at both dose levels. However, the incidence of carcinomas in males at 850 ppm was within the reported historical control incidence. In female mice, valifenalate appeared to be less potent with a smaller, but statistically significant, increase in adenomas only being reported at a dose level of 5000

ppm. The incidence of adenoma was 4 and 10% at 850 and 5000 ppm, respectively. At both dose levels, this incidence was outside the historical control incidence.

Investigative study: Comparison of C57BL/6 mice and CD1 mice to determine if C57BL/6 mice are a suitable strain for a subsequent study in peroxisome proliferator-activated receptor-alpha (PPARα) knock out mice derived from C57BL/6 strain (confidential study number 68)

Two strains of mice (5 males/group) were fed with 7000 ppm valifenalate (purity 99.68%) in diet during days. Several hepatic parameters were determined and compared with controls of respective strain non-exposed to valifenalate. The results are shown below:

	CD1	**C57Bl/6**
Absolute Liver weight	↑ 19.5%	↑ 13.8%
Relative liver weight	↑ 21%	↑ 16%
PCoA oxidation	↑ 1.6 fold	↑ 1.9 fold
Hepatic pentoxyresorufin-O-depentylation (PROD)	↑ 2.1 fold	↑ 3.4 fold
Hepatic 12-hydroxylauric acid	↑ 4.9 fold	↑ 7.1 fold

Overall, the DS concluded that the response in both strains was very similar. It was concluded that the C57BL/6 mouse strain is an appropriate background strain for further investigations using the PPARα knockout model

Investigative study: Comparison of response in PPARα knockout mice with wild type controls (confidential study number 69)

C57BL/6 wild type and PPARα knock out CD1 mice (10 males/group) were fed with 7000 ppm valifenalate (purity 99.68%) in diet during 7 and 14 days. Several hepatic parameters were determined and compared with controls of respective strains non-exposed to valifenalate. The results are shown below:

	C57BL/6 wild type		**PPARα knock out CD1**	
	7 days	14 days	7 days	14 days
S-phase	↑ 8.2 fold	↑ 3.5 fold	↑ 5.4 fold	↑ 1.9 fold
Liver pathology:				
↑ minimal to mild centrilobular hypertrophy	10/10	-	2/10	-
moderate centrilobular hypertrophy	-	10/10	-	-
increased mitosis	-	6/10	-	-
PCoA oxidation	-	↑ 2.0 fold	-	↑ 1.3 fold
Acox1 mRNA	-	↑ 1.8 fold	-	↑ 1.3 fold
12-hydroxylauric acid levels	-	↑ 7.7 fold	-	↑ 4.0 fold
Cyp2b10 mRNA level	-	↑ 50 fold	-	↑ 50 fold
PROD activity	-	↑ 6.0 fold	-	↑ 7.1 fold
Cyp3a11 mRNA levels	-	↑ 6.3 fold	-	↑ 8.5 fold

Overall, the DS concluded that PPARα pathway is responsible for a portion of the hepatic response, and additional mechanisms mediated by CAR and PXR activation are also involved. RAC also notes that, despite hepatocellular hypertrophy was clearly lower in knock-out mice than in wild mice, there was no significant differences between the wild type and knock out mice in the level

of expression of the biomarker of activation of PPAR receptor (Acox1 mRNA level). Moreover, RAC also notes that the level of activation of CAR (Cyp2b10 mRNA level) and PXR (Cyp3a11 mRNA levels) was quite comparable.

Investigative study: Investigate the potential of valifenalate to activate CAR and/or PPARα nuclear hormone receptors and stimulate cell proliferation in isolated hepatocytes (confidential study number 70)

Mouse hepatocytes from CD1 strain were exposed to valifenalate (purity 99.68%), phenobarbital and WY-14.643 as positive controls. Valifenalate 300 µM (a concentration able to reduce the ATP levels by 74%) and also 100 µM (a non-cytotoxic concentration) caused no impact on any of the biochemical marker assessed. However, the positive controls increased DNA synthesis, the mRNA levels of Cyp2b10, Cyp4a10, Cyp4a14c, Cyp4a10, Cyp4a14, Cyp2b10 and Acox1, PCoA oxidation and PROD activity.

Overall, the DS concluded that valifenalate does not activate either mouse CAR or PPARα when assessed *in vitro* as demonstrated by the lack of hypertrophic and hyperplasic responses in the CD-1 mouse hepatocytes.

Investigative study: Investigation of mechanism of possible liver toxicity. Assessments included cell proliferation, CYP enzymes (activity and/or mRNA expression), peroxisomal β-oxidation, catalase histochemistry and oxidative stress (TBARS) (confidential study number 66)

Crl:CD-1 mice (18 males/group) were dosed with 21, 249 and 1050 mg/kg bw/day valifenalate (purity 97.83%) or phenobarbital as positive control during 14 days. Several hepatic parameters were determined and compared with controls of respective strains non-exposed to valifenalate. The results are shown below:

	Dose valifenalate (mg/kg bw/day)		
	21	249	1050
Cyp4a-1 enzyme sub family (Lauric acid 12-hydroxylase)	No effects	↑ 408%	↑ 1106%
Peroxisomal β-oxidation	No effects	↑ 208%	↑ 308%
Relative liver weight	No effects	↑ 13%	↑ 35%
Hepatocellular hypertrophy	No effects	4/6	6/6
Cyp1a1 mRNA level	↓ 0.8 fold	↑ 1.2 fold	↑ 1.2 fold
Cyp1a2 mRNA level	↓ 0.7 fold	↓ 0.8 fold	↓ 0.3 fold
Cyp2b10 mRNA level	↑ 1.6 fold	↑6.2 fold	↑20
Cyp3a11 mRNA level	↑ 1.1 fold	↑ 6.1 fold	↑9.5 fold
Catalase	↑ 6% fold	↑ 12%	↑ 16%

	Dose phenobarbital (mg/kg bw/day)
	130
Cyp 2B10 mRNA level	↑223 fold
Cyp3a11 mRNA level	↑12 fold
Cyp1a1 mRNA level	↑3.6 fold
Cyp1a2 mRNA level	↑2.9 fold
Peroxisomal β-oxidation	No increase
Relative liver weight relative to body weight	↑ 55% by day 3, 37% by day 14
Hepatocellular hypertrophy	↑ 6/6 after 3 and 14 days, severity more marked after 14 days
Catalase	No increase

Overall, the DS concluded that valifenalate appears as moderate and dose dependent liver enzyme inducer of the peroxisomal-proliferator type and that the mode of action as a liver enzyme inducer of the polycyclic aromatic hydrocarbon-, steroid-, or phenobarbital-type can be excluded.

Summary of mechanistic studies on liver effects

The data from these studies have been considered in detail by the DS (see Annex II to the CLH-report) and were summarised below in the section Supplemental information. These mechanistic studies allowed considering a mode of action for the carcinogenic effects of valifenalate with an initiating event based on the co-activation of multiple nuclear receptors, CAR/PXR/PPARα, and as a direct consequence, the associated induction of gene expression and enzyme activity of Cyp2b10, Cyp3a11 and Cyp4a.

The second key event is the increased hepatocellular proliferation and is also initiated in CD-1 mice exposed to valifenalate, on a time scale not dissimilar to the appearance of induction of the hepatic metabolising enzymes.

The final key event is the longer-term formation of carcinomas via the development of altered, hyperplastic, hepatic, foci and the subsequent development of benign and, ultimately, malignant hepatocellular neoplasms.

Comparison with the criteria

Classification in category 1A concerns substances known to have carcinogenic potential for humans and is largely based on human evidence. Since there are no human data it cannot be concluded that valifenalate has known carcinogenic potential for humans; therefore Category 1A is not applicable.

Category 1B is for substances with sufficient evidence of carcinogenic potential for humans. For that, increases incidences of malignant neoplasms or an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under GLP, can also provide sufficient evidence. In the case of valifenalate, the database contains one study showing increment of malignant lesions in a single species and sex and therefore the conditions for category 1B are not met.

Category 2 is reserved for substances with evidences of carcinogenicity not sufficiently convincing to place the substance in Category 1A or 1B and can be set if the evidence of carcinogenicity is restricted to a single experiment, as is the case of valifenalate.

A full range of investigative studies was included in the CLH-dossier to determine the mode of action of valifenalate in the mouse. These experiments show that liver effects are initiated by activation of receptors CAR, PXR and PPARα and it was concluded that these effects were not likely to occur in humans on a quantitative basis.

RAC recognises that the mechanism of action proposed by the DS (nuclear receptor activation → increase of replicative DNA synthesis → hypertrophy → carcinogenesis) is plausible. However, RAC also notes that the database is not robust enough for rule out the relevance of valifenalate-induced hepatocarcinomas in humans. RAC notes the following concerns:

- Weak (up to 3.6 times) increases in the expression of Cyp1a1 and Cyp1a2 were reported after dosing CD-1 mice for 14 days with 850 ppm valifenalate (Table A1 in Annex 2 to the CLH-report); while the level of expression of these Cyp at 7000 ppm (dose at which most of other mechanistic studies were performed) is unknown. It suggests that a potential role of AhR in the mechanism of action cannot be totally ruled out.

- Inconsistencies detected in the study with PPAR-α mice, where, moreover, lack of positive control was detected
- Lack of data with CAR/PXR knock-out mice
- Lack of data with human hepatocytes
- Fails in the valifenalate to induce *in vitro* changes in biochemistry of hepatocytes without evidences that hepatocytes were not metabolically competent
- Cytoplasmic eosinophilia in hepatocytes in the 1.5-year study in mouse, in the 28-days and 90-days toxicity studies in dogs; hepatocyte and liver macrophage pigmentation in the 1.5-year study in mouse; liver cell necrosis in the 28-day study in dogs and pale cytoplasm in dog hepatocytes in the in the 90-day study and 52-week study suggest cytotoxicity; which could be a carcinogenic mode of action alternative to the proposed PPAR activation.

Overall, there is insufficient evidence to support the non-relevance of the observed liver tumours for humans and therefore RAC supports the **classification of valifenalate as Carc. 2, H351; "Suspected of causing cancer".**

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

DS proposed no classification of valifenalate for sexual function and fertility, development and lactation based on lack of effects detected in a 2-generation reproduction toxicity study, one developmental toxicity study in rats and one developmental toxicity study in rabbits.

Comments received during consultation

One MSCA supported the proposal of no classification for adverse effect on sexual function and fertility, development and lactation but demanded discussion about the effects on reproductive organs found in some of the repeated dose toxicity studies. The DS provided such discussion and the arguments (supported by RAC) are incorporated into the discussion below.

This same MSCA also requested discussion about the lack of *corpora lutea* and decreased absolute and ovary/brain ratio seen in the F1 parental generation from the high dose of the OECD TG 416 study. The DS replied that the lack of corpora lutea in the parental F1 generation of the 2-generation rat study cannot be confirmed because no difference between the high dose and control group occurred, which indicates a no test item-related effect. Likewise, the mentioned decreased absolute and ovary/brain ratios in the F1 parental generation from the high-dose group cannot be confirmed since the organ/body weight ratios of the ovaries were 0.021, 0.020, 0.022 and 0.020 (ovaries right) and the organ/brain weight ratios 3.177, 2.830, 3.039 and 2.854 (ovaries right) in the order of the ascending doses. They were clearly not affected by the treatment.

A second MSCA also commented that the exclusion of the litter with total loss of pups is not justified. This same MSCA demanded to incorporate into the CLH-report the incidence of the findings "no milk in stomach" as reported in the Annex 1. Finally, this MSCA also raised the opinion that a need for classification regarding developmental toxicity effects or effects on/via lactation because of reduced pup survival. The DS provided the data from litter with total loss (incorporated in the discussion below) and indicated that this data were initially removed because the incidence of dams with total litter loss were not dose-related; which suggests that a relationship with the treatment is very unlikely. Nevertheless, the inclusion of this data (see

below) does not alter the main conclusion since no dose-response was observed for all assessed parameters and in most of the cases, the results at the top dose were covered by the HCD. As regard the finding "no milk in stomach" the DS highlighted that no clear dose-response in this parameter was noted with regard to the litter incidence and therefore these findings can be included within the biological variability. Overall, the DS considered that the discussed viability and weaning indices of the F1 generation would be within the HCD and is unlikely that the treatment had an effect on these parameters, especially considering the fact that no effects were detected in the P generation. Thus, the DS maintained the proposal of no classification for reproductive toxicity.

One manufacturer/company agreed with the DS's proposal for no classification.

Assessment and comparison with the classification criteria

Fertility and sexual function

The reproductive toxicity of valifenalate was investigated in a 2-generation reproduction toxicity study in rats. Additionally, some data about effects on sexual organs were reported in several repeated dose toxicity studies.

2-generation reproduction toxicity study in rats (Confidential study number 27)

The study was conducted according current OECD TG 416 and observing GLP. Rats (24/sex/group) were treated with 0, 1250, 4300 or 15000 ppm (reduced to 0, 850, 2900 or 10000 ppm during lactation) valifenalate in laboratory animal diet. Mean achieved test item intakes were as shown below:

		1250 ppm (mg/kg bw/day)	4300 ppm (mg/kg bw/day)	15000 ppm (mg/kg bw/day)
P generation				
Males	Pre-pairing	80.8	277.4	986.3
	After pairing	61.4	216.1	757.9
Females	Pre-pairing	92.7	318.8	1150.3
	Lactation	79.2	273.2	992.8
	Lactation	123.9	408.4	1384.0
F1 generation				
Males	Pre-pairing	83.5	294.2	1024.8
	After pairing	63.8	216.3	763.8
Females	Pre-pairing	93.0	326.1	1145.6
	Gestation	84.1	295.5	1030.8
	Lactation	129.2	429.3	1383.3

The main results and observations in this study are discussed below.

Parental toxicity

See Table 5 above. The main remarkable effects were increases in relative liver weight and liver and thyroid hypertrophy in both P and F1 together with slight clinical signs (ruffled fur) on F1.

Offspring toxicity

No treatment related effects on F1a at any dose were noted.

No treatment related effects at the lowest dose were noted on F2a. The main effects on this F2a at higher doses were:

	4300 ppm (2900 ppm)		15000 ppm (10000 ppm)	
	M	F	M	F
Pup weight gain (days 0-21)	↓ 9%	↓ 9%	↓ 8%	↓ 8%
Absolute spleen weights (no histological correlate)	↓ 26%	↓ 26%	↓ 18%	↓ 23%
Relative spleen weights (no histological correlate)	↓ 20%	↓ 17%	↓ 12%	↓ 17%
Glycogen deposition liver	16/19 (severity 2.1) vs 20/20 (severity 2.5) controls	14/18 (severity 1.6) vs 20/21 (severity 1.7) controls	18/22 (severity 1.5) vs 20/20 (severity 2.5) controls	14/21 (severity 1.3) vs 20/21 (severity 1.7) controls

RAC noted that glycogen deposition liver was not dose-related and therefore cannot be considered treatment related. No histopathological alterations were noted in spleen and therefore the alterations in spleen weight were not considered relevant.

Reproductive toxicity

No reproductive effects were noted on P generation.

In F1, three dams of the mid dose and one dam of the top dose suffered total litter loss. Next table offers an overview of relevant parameters in regard to pup mortality and survival:

	Dose (ppm)				
Parameter	0	1250/850	4300/2900	15000/10000	HCD[1]
All dams					
Pup loss days 0-4 p.p. (total number)	18	8	35	39	0-23
Pup loss days 0-4 p.p. (% of living pups)	7.4	3.2	14.8	15.2	0-8.5
Mean no. postnatal loss/litter days 0-4 p.p.	0.9	0.3	1.5	1.7	0-1.0
Mean living pups/litter day 4 p.p.	7.7	7.7	6.7	7.1	7.1-8.0
Mean living pups/litter day 21 p.p.	7.5	7.7	6.3	6.7	6.8-8.0
Mean pup loss/litter day 21	0.19	0.04	0.39	0.43	0-1.2
Without dams with total litter loss					
Pup loss days 0-4 p.p. (total number)	18	8	11	25	0-23
Pup loss days 0-4 p.p. (% of living pups)	7.4	3.2	5.5	9.6	0-8.5
Mean no. postnatal loss/litter days 0-4 p.p.	0.9	0.3	0.6	1.7	0-1.0
Mean living pups/litter day 4 p.p.	7.7	7.7	7.8	7.5	7.1-8.0
Mean living pups/litter day 21 p.p.	7.5	7.7	7.7	7.0	6.8-8.0
Mean pup loss/litter day 21	0.19	0.04	0.10	0.43	0-1.2
% Viability index	92.6	96.8*	95.5	90.4	91.5-100
% Weaning index	97.5	99.4	98.6	93.9	84.5-100

[1] Historical control data from 10 studies conducted from May 2002 to December 2007 (current study started November 2002)

RAC noted that no dose-response was observed in the effect total litter loss (no incidence at the lowest dose, 3 dams at the mid dose and 1 dam at the top dose). It suggests that this effect can be incidental and not treatment related.

When all dams were considered, the total number of pup loss on days 0-4, percentage of living pups on days 0-4 and mean number of post-natal loss on days 0-4 in the mid and top dose was higher than HCD. These parameters were higher than HCD only at the top dose when dams with total litter loss were removed. Nevertheless, RAC noted that dose-response was not observed in these parameters since the increment of dose of 3.4 times between mid and high dose barely has effect on incidence. By the other hand, no negative effects on survival is evident since the records for mean living pups/litter day on days 4 and 21 and mean pup loss/litter day 21 were (in both cases with all dams and without dams with total litter loss) were covered by the HCD.

Effects on sexual organs in the repeated dose toxicity studies

Repeated dose toxicity studies in dogs showed certain effects on sexual organs (Table 7). These effects were mainly immaturity in prostate gland and reductions in weights of testis, epididymis and ovaries.

The findings on prostate glands are relatively common in short-term studies in dogs. Reductions in prostate gland weights were reported in all three studies in dogs. However, these reductions were noted in some cases also in control group or even in all animals of all groups. These findings, together with the small group size (3-4 animals/group) that bias the assessment of dose-response and the lack of alteration with histopathological correlation in the 52-week study suggest that prostate gland alterations cannot be addressed to valifenalate effects.

Reductions in testis, ovary and epididymis weights were also reported in these studies in dogs. However, these reductions were not correlated with histopathological changes and therefore are not considered by RAC as toxicologically relevant, especially considering that these effects were not reported in mice and rats.

Development

Table 12 summarises the available developmental toxicity studies with valifenalate.

Table 12: *Summary for animal studies on developmental toxicity with valifenalate*.

Method	Results	Reference
Developmental toxicity OECD TG 414 (2001) GLP Oral (gavage) Rat Crl:CD(SD)BR 25 mated females/group Valifenalate (IR5885) Purity: 98.9%	Maternal toxicity 1000 mg/kg bw/day: No treatment related adverse effects at any dose Developmental toxicity **No treatment related adverse effects.** Incidences of corpora lutea, implantations, pre-implantation losses, post implantation losses, mean foetal weight, foetuses with external malformations, foetuses with skeletal malformations and foetuses with visceral malformations in all cases not statistically different from concurrent controls and within HCD	Confidential study number 9

0, 100, 300 and 1000 mg/kg bw/day Dosing on gestation days 6-19 Vehicle: 0.5% MC		
Developmental toxicity OECD 414 (2001) GLP Oral (gavage) Rabbit NZW (HY/CR) 22 mated females/group Valifenalate (IR5885) Purity: 98.9% 0, 100, 300 and 1000 mg/kg bw/day Dosing on gestation days 6-28	<u>Maternal toxicity</u> 1000 mg/kg bw/day: No treatment related adverse effects <u>Developmental toxicity</u> **No treatment related adverse effects.** Incidences of corpora lutea, implantations, pre-implantation losses, post implantation losses, dead foetuses, mean foetal weight, foetuses with external malformations, foetuses with skeletal malformations, and foetuses with visceral malformations in all cases not-statistically different from concurrent controls and within HCD	Confidential study number 10

Lactation

The two-generation study of valifenalate in rats has already been described. The dietary concentrations were lowered for the lactation period as an attempt to maintain the level of test item intake. Nevertheless, mean achieved dose levels were increased above pre-pairing levels (approximately 124, 408 and 1384 mg/kg bw/day in the low, mid and high dose groups respectively cf. 80, 277 and 986 mg/kg bw/day). Parental toxicity was observed at mid and high doses in all generations. Increased neonatal loss, reduced viability indices and increased pup mortality was seen in the F1 litters in the mid and high dose. There were no other treatment related adverse effects on the offspring.

The incidence of the finding 'no milk in stomach' was increased in the mid dose and high dose groups, but with regard to the litter incidences 1/21, 1/23, 6/23 and 4/23 in ascending order of doses. No clear relationship with doses could be established and this was most likely due to variability. Such findings, including cannibalism are background findings, which often occur in reproductive toxicity studies as non-treatment-related phenomenon. It is consistent with the fact that this observation was also made in the control group in this study and it occurred mainly in the litters with the mentioned losses, where the possibility of milk uptake by pups was apparently limited. There is no evidence of treatment-related impairment of the nursing behaviour of the dams.

The discussed viability and weaning indices of the F1 generation would be within the HCD if the dams with total litter loss were taken out of the evaluation, as can be seen in the table above.

Therefore, the treatment has unlikely had an effect on these parameters, which is further supported by the fact that no effects on these parameters occurred in the P generation.

Comparison with the criteria

Sexual function and fertility

No effects on reproductive performance parameters and reproductive performance could be attributed to valifenalate. Therefore, RAC supports the DS's proposal for **no classification of valifenalate for adverse effects on sexual function and fertility.**

Development

In rat and rabbit prenatal developmental toxicity studies of valifenalate, no treatment related maternal toxicity was demonstrated at the limit dose of 1000 mg/kg bw/day and there was no evidence of developmental toxicity or of teratogenicity in either species. There were no treatment related effects on development of the offspring in the 2-generation toxicity study in rats rat to warrant classification of valifenalate as a known, presumed or suspected human reproductive toxicant, especially considering that the effects on pup loss days 0-4 are not considered by RAC robust enough because they were not noted in P litters. Therefore, RAC supports the DS's proposal for **no classification of valifenalate for development.**

Adverse effects on or via lactation

There was no indication of impaired nursing behaviour during lactation. The results of the study do not indicate any direct, primary adverse effect on the offspring due to transfer of the chemical via the milk or to the quality of the milk. Thus, RAC supports the DS's proposal for **no classification of valifenalate for adverse effects on or via lactation.**

RAC evaluation of aspiration toxicity

Summary of the Dossier Submitter's proposal

DS proposed no classification of valifenalate for aspiration toxicity based on data lacking.

Comments received during consultation

No comments were received during consultation.

Assessment and comparison with the classification criteria

RAC notes that the hazard class aspiration toxicity is not relevant for solids and therefore **supports no classification for valifenalate**.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The Dossier Submitter (DS) proposed to classify the substance as Aquatic Chronic 2; H411 based on lack of rapid degradation and a 96h nominal NOEC value of 0.106 mg/L for the marine diatom *Skeletonema costatum*.

Degradation

A hydrolysis study according to OECD TG 111 and in compliance with GLP was run at pH 4, 7 and 9 in the dark in aqueous buffered solutions. Valifenalate was stable at pH 4 (50°C), while at pH 7 and pH 9 a pseudo-first order kinetic hydrolysis reaction was observed. The following DT_{50} values of 90.94 d (25ºC), 7.62 d (50ºC), 5.21 d (55ºC) and 2.09 d (65ºC) at pH 7 and 4.15 d (25ºC) and 0.33 d (50ºC) at pH 9 were determined. The hydrolytic degradation of valifenalate increased with higher pH values. Two main compounds found were the unchanged parent substance valifenalate and IR5839 (3-(4-chlorophenyl)-3-({(2S)-2-[(isopropoxycarbonyl) amino]-3-methylbutanoyl} amino) propanoic acid, also referred to as IR5885 acid). For both of the compounds the diasteroisomeric ratio (S,R/S,S) was approximately 1:1.

Photochemical degradation in water was not expected to be significant since the molar absorption coefficient (ε) is <10 M-1 × cm-1 at λ >290 nm.

There was one ready biodegradability test available for valifenalate following EEC method C.4-D (1992) and OECD TG 301F (Manometric Respirometry) and in compliance with GLP using domestic activated sludge (adaptation not specified) that resulted in 3% (based on $ThOD_{NH4}$) and 2% (based on $ThOD_{NO3}$) degradation after 28 days.

A water/sediment study carried out according to OECD TG 308 and in compliance with GLP, was conducted using two aquatic systems (Pond and River systems) for 22 days. The radioactivity in the surface water decreased during all the study and it was 40.84% (Pond) and 43.74% (River) of applied radioactivity (AR) at the end of incubation period. The radioactivity in the sediment increased throughout the study reaching 50.64% AR (Pond) and 45.51% AR (River) at the end of incubation period. Valifenalate degraded in both aquatic systems: after 22 days it accounted for 5.92% AR (Pond) and 5.51% AR (River). In the whole system, the DT_{50} values were 4.5 days (Pond) and 4.71 days (River) and DT_{90} values, 14.9 days (Pond) and 15.64 days (River). Six compounds were found in the surface water and in the sediment extracts. The main degradation products were S2 and S3: S2 reached 52.80% AR (Pond) and 56.34% AR (River). S2 was identified as 3-(4-chlorophenyl)-3-({(2S)-2-[(isopropoxycarbonyl) amino]-3-methylbutanoyl} amino) propanoic acid (also referred to as IR5839 or IR5885 acid). The compound S3, that increased up to a maximum of 13.77% AR (Pond) and 8.16% AR (River), was identified as 4-chlorobenzoic acid (also referred to as PCBA). The fraction S6 slowly increased reaching 8.93% AR and 8.04% AR. It was represented by a pool of 4 compounds and none of these reached values higher than 3.13% AR. None of the other compounds, S4 and S5, ever reached levels higher than 5% AR. The non-extractable radioactivity (bound residue) increased to 8.99% AR (Pond) and 16.24% AR (River). The radioactivity in the ^{14}C-CO_2 traps was always lower than the detection limit in both the systems except at the last three sampling times when it reached values ranging between 0.77% AR and 1.24% AR. The ^{14}C-Mass Balance was always higher than 90% AR and ranged from 90.61% to 104.12% AR for Pond system and from 90.49% to 107.96% AR for River system.

In conclusion, the DS considered valifenalate not to be rapidly degradable for classification purposes.

Bioaccumulation

A bioconcentration study (OECD TG 305, GLP) was available for valifenalate. Rainbow trout (*Oncorhynchus mykiss*) was exposed to concentrations (93.5 and 893.5 µg/L) of the radiolabelled valifenalate for 14 days in a flow-through system, followed by 14-day depuration period in clean water. Due to the extremely low accumulation of valifenalate in fish at both dose levels, no relevant plateau levels and consequently no half-lives or accumulation/depuration kinetics could be determined. Based on the total radioactivity concentration in the exposure water and the residual radioactivity found in fish parts, ratios between fish and water (BCF) amounted to 1.3, 3.0 and 2.3 for edibles, non-edibles and whole fish, respectively, indicating lack of bioconcentration at both dose levels. The kinetic BCF (growth corrected and lipid-normalized) was < 4 for whole fish. Analyses of radioactivity of the test water showed mainly the presence of the parent compound at both dose levels throughout the entire exposure period. Besides the constant levels of parent compound ranging on average from 96.2 to 98.0% of the radioactivity recovered, three unknown radioactive fractions W0, W2 and W3/4 were found in minor amounts (< 3% of the radioactivity recovered).

Furthermore, the measured octanol-water partition coefficient (log K_{OW}) determined according to OECD TG 117 (HPLC method) is 3.05 – 3.11 at 20°C and pH 7.

The DS concluded that valifenalate has a low potential to bioconcentrate and is therefore not considered a bioaccumulative substance for classification purposes.

Aquatic Toxicity

The DS provided aquatic toxicity data for the active substance regarded as reliable in the CLP Report, and a summary of the relevant information on aquatic toxicity is provided in the following table (the key endpoints used in hazard classification are highlighted in bold).

Data for sediment-dwelling invertebrates (marine amphipod *Leptocheirus plumulosus* and freshwater midge *Chironomus dilutes*) were reported in CLH report but were not used for classification because the endpoint values were presented in relation to sediment concentrations of valifenalate (mg/kg).

Table: *Summary of relevant information on aquatic toxicity of valifenalate*

Method	Species	Endpoint	Toxicity value (mg/L)	Reference
Short-term toxicity				
OECD TG 203	*Oncorhynchus mykiss*	96h LC_{50} (mortality)	>100 nom	Anonymous (2003b), final results: Anonymous (2003a)
OECD TG 203	*Brachydanio rerio*	96h LC_{50} (mortality)	>100 nom	Anonymous (2003), final results: Anonymous (2003)
US EPA OPPTS 850.1075	*Cyprinodon variegatus*	96h LC_{50} (mortality)	>15 mm	Anonymous (2005a)
US EPA OPPTS 850.1075	*Lepomis macrochirus*	96h LC_{50} (mortality)	>40 nom	Anonymous (2015a)

Method	Species	Endpoint	Toxicity value (mg/L)	Reference
OECD TG 202	*Daphnia magna*	48h EC_{50} (immobilization)	>100 nom	Anonymous (2002), final results: Anonymous (2002)
US EPA OPPTS 850.1035	*Americamysis bahia*	**96h LC_{50}** (mortality)	**2.8 mm**	Anonymous (2005c)
US EPA OPPTS 850.1025	*Crassostrea virginica*	96h EC_{50} (shell deposition)	3.1 mm	Anonymous (2005d)
OECD TG 201	*Scenedesmus subspicatus*	72h E_bC_{50} 72h E_rC_{50} (growth)	>100 nom >100 nom	Anonymous (2002b), final results: Anonymous (2002)
US EPA OCSPP 850.4500	*Skeletonema costatum*	96h I_bC_{50} 96h I_rC_{50} 96h I_yC_{50} (growth)	>9.48 gmm >9.48 gmm >9.48 gmm	Hicks (2015b)
US EPA OCSPP 850.4500	*Navicula pelliculosa*	96h I_bC_{50} 96h I_rC_{50} 96h I_yC_{50} (growth)	>5.45 gmm >5.45 gmm >5.45 gmm	Bergfield (2015a)
US EPA OCSPP 850.4550	*Anabaena flos-aquae*	96h I_bC_{50} 96h I_rC_{50} 96h I_yC_{50} (growth)	>4.13 gmm >4.13 gmm >4.13 gmm	Aufderheide (2015b)
US EPA OCSPP 850.4400	*Lemna gibba*	7d EC_{50} (growth)	>5.02 gmm	Bergfield (2015b)
Long-term toxicity				
OECD TG 215	*Oncorhynchus mykiss*	28d NOEC (growth)	≥100 nom	Anonymous (2003c), final results: Anonymous (2003b)
EPA OPPTS 850.1400	*Pimephales promelas*	33d NOEC (growth)	12 nom	Anonymous (2005b)
OECD TG 211	*Daphnia magna*	22d NOEC (reproduction) 22d NOEC (mortality)	3.2 nom 10 nom	Anonymous (2003d), final results: Anonymous (2002)
OECD TG 201	*Scenedesmus subspicatus*	72h NOEC (growth)	≥100 n	Anonymous (2002b), final results: Anonymous (2002)
US EPA OCSPP 850.4500	*Skeletonema costatum*	**96h NOEC** (growth)	**0.106 gmm**	Hicks (2015b)
US EPA OCSPP 850.4500	*Navicula pelliculosa*	96h NOEC (growth)	5.45 gmm	Bergfield (2015a)

Method	Species	Endpoint	Toxicity value (mg/L)	Reference
US EPA OCSPP 850.4550	*Anabaena flos-aquae*	96h NOEC (growth)	2.15 gmm	Aufderheide (2015b)
US EPA OCSPP 850.4400	*Lemna gibba*	7d NOEC 7d EC_{10} (growth)	5.02 gmm > 5.02 gmm	Bergfield (2015b)

Note: nom – nominal concentrations; mm – mean measured concentrations; gmm - geometric mean measured concentrations;

Acute toxicity

For acute aquatic toxicity, reliable toxicity data for the active substance were reported for fish, invertebrates, algae and aquatic plants, with invertebrates being the most sensitive trophic level. The lowest acute toxicity value is the 96h mean measured LC_{50} of 2.8 mg/L for saltwater mysid shrimp *Americamysis bahia* which is above the classification threshold value of 1 mg/L. Therefore, the DS proposed **not to classify** the valifenalate as acutely hazardous to the aquatic environment.

Chronic toxicity

For chronic aquatic toxicity, reliable toxicity data for the active substance were reported for fish, invertebrates, algae and aquatic plants, with algae being the most chronically sensitive group. The lowest chronic toxicity value is the 96h nominal NOEC of 0.106 mg/L for marine diatom *Skeletonema costatum.* The DS proposed to classify the substance as **Aquatic Chronic 2** based on the lowest chronic endpoint for algae and considering that the substance is not rapidly degradable and has low potential for bioaccumulation.

Comments received during consultation

Comments were received from three Member States (MS) and one company-manufacturer. Two MSs and the company-manufacturer agreed with DS proposal to classify the substance as Aquatic Chronic 2. The third MS agreed with the proposed classification but based on a different interpretation of the data. The MS pointed out the limitations of the key chronic toxicity study on algae *Skeletonema costatum* (Hicks, 2015) and that, due to these limitations of the key study, the MS was of the opinion that the study does not support the proposed classification. In the view of the MS, the classification should be based on the surrogate approach for the most acutely sensitive endpoints (saltwater mysid *Americamysis bahia*), which would result in the same classification as proposed by DS. The DS disagreed with the commenting MS and is of the opinion that the algae study should be used for classification. As regards the application of the surrogate approach, the view of the DS is that this approach is not warranted since a sufficient set of chronic studies is available.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS's proposal to consider valifenalate as not rapidly degradable. Valifenalate is hydrolytically stable at pH 4 but it undergoes hydrolysis with increasing alkalinity. Hydrolysis DT_{50} values at pH 7 are 90.94 d (25ºC), 7.62 d (50ºC), 5.21 d (55ºC) and 2.09 d (65ºC) and pH 9 are 4.15 d (25ºC) and 0.33 d (50ºC). Two main compounds were found, unchanged parent substance and IR5839. Data on hydrolysis might be considered for classification purposes only when the longest half-life determined within the pH range 4-9 is less than 16 days (corresponding to a degradation of > 70% within 28 days). Accordingly, valifenalate is hydrolytically stable.

In a 28-day ready biodegradability study following OECD TG 301F (GLP), 3% degradation was observed, indicating that valifenalate is not readily biodegradable.

The results of the aerobic water/sediment simulation study showed degradation of the valifenalate in both aquatic systems (5.92% AR (Pond) and 5.51% AR (River) after 22 days). In addition, rapid loss of the valifenalate from the whole system was observed (DT_{50} values were 4.5 days (Pond) and 4.71 days (River) and DT_{90} values, 14.9 days (Pond) and 15.64 days (River)). Six degradation products were formed in water and sediment. The main metabolites were IR5839, PCBA and fraction S6. No information on toxicity of the metabolites to allow classification of the metabolites is available in the CLH report.

Overall, although valifenalate degrades quickly in the whole system of the water/sediment study, the substance does not pass the ready biodegradability test, the available abiotic and biotic degradation information does not indicate that valifenalate is ultimately degraded (> 70%) within 28 days (equivalent to a half-life < 16 days) or transformed to non-classifiable metabolites. Consequently, RAC considers the substance to be not rapidly degradable for the purposes of environmental classification.

Bioaccumulation

RAC agrees with the DS that valifenalate has a low potential to bioaccumulate in aquatic organisms. The basis for this is that measured BCF values of < 4 is below the CLP criterion of 500 and the measured log K_{ow} value of 3.05 – 3.11 is below the CLP criterion of 4.

Acute toxicity

RAC is of the opinion that adequate acute toxicity data are available for fish, invertebrates, algae and aquatic plants. Invertebrates are the most sensitive group and the lowest result is a 96h EC_{50} value of 2.8 mg/L for mysid shrimp *Americamysis bahia*. RAC notes that all acute toxicity endpoints ($L(E)C_{50s}$ and IC_{50}) for fish, invertebrates, algae and aquatic plants (see table) are above the threshold value of 1 mg/L. Consequently, RAC concludes that **valifenalate does not warrant classification for acute aquatic toxicity**.

Chronic toxicity

RAC is of the opinion that reliable long-term aquatic toxicity data are available for all three trophic levels. The lowest chronic effect value corresponds to a test with *Skeletonema costatum* with a 96h NOEC of 0.106 mg/L. As the value is >0.1 but <1 mg/L and the substance is considered not rapidly degradable, RAC concludes that following table 4.1.0(b)(i) of CLP, a classification as Aquatic Chronic 2 (H411) is warranted.

RAC notes that no chronic toxicity test data are available for the most sensitive species under acute testing (*Americamysis bahia*). Using table 4.1.0(b)(iii) of CLP, considering that Valifenalte

is not rapidly degradable, the 96h LC_{50} of 2.8 mg/L indicates classification as Aquatic Chronic 2, which supports the outcome derived using chronic data.

In summary, RAC agrees with the DS that **valifenalate warrants classification as Aquatic Chronic 2 (H411).**

RAC evaluation of hazards to the ozone layer

Summary of the Dossier Submitter's proposal

Pure valifenalate has a vapour pressure of 9.6×10^{-8} Pa (20°C) and water solubility of 24.1 mg/L (20°C) resulting in a calculated Henry's Law constant of 1.6×10^{-6} Pa m^3/mol (20°C, pH 5.4 ± 0.5). This combination of properties indicates no volatilisation and, thus, no significant amounts of valifenalate are to be expected in air. The Atkinson calculated oxidative photochemical degradation half-life is 7.5 hours assuming a hydroxyl radical concentration of 5×10^5 molecules/cm^3 (Fisk, 2003).

Comments received during consultation

One comment was received from company-manufacturer which agreed with DS proposal not to classify the substance as hazardous to the ozone layer.

Assessment and comparison with the classification criteria

Transport of valifenalate in air is considered to be negligible due to its very low vapor pressure and Henry's constant, whilst its photochemical oxidative degradation in air is expected to be rapid. Therefore, exposure of stratospheric ozone to valifenalate is expected to be negligible.

Thus, RAC agrees with the DS's proposal that **no classification is warranted for this hazard class.**

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).